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Modeling the effects of temperature and pH on the resistance of *Alicyclobacillus acidoterrestris* in conventional heat-treated fruit beverages through a meta-analysis approach

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ABSTRACT

In this work, all publicly-accessible published findings on *Alicyclobacillus acidoterrestris* heat resistance in fruit beverages as affected by temperature and pH were compiled. Then, study characteristics (protocols, fruit and variety, °Brix, pH, temperature, heating medium, culture medium, inactivation method, strains, etc.) were extracted from the primary studies, and some of them incorporated to a meta-analysis mixed-effects linear model based on the basic Bigelow equation describing the heat resistance parameters of this bacterium. The model estimated mean D^* values (time needed for one log reduction at a temperature of 95 °C and a pH of 3.5) of *Alicyclobacillus* in beverages of different fruits, two different concentration types, with and without bacteriocins, and with and without clarification. The z_T (temperature change needed to cause one log reduction in *D*-values) estimated by the meta-analysis model were compared to those ('observed' z_T values) reported in the primary studies, and in all cases they were within the confidence intervals of the model. The model was capable of predicting the heat resistance parameters of *Alicyclobacillus* in fruit beverages beyond the types available in the meta-analytical data. It is expected that the compilation of the thermal resistance of *Alicyclobacillus* in fruit beverages, carried out in this study, will be of utility to food quality managers in the determination or validation of the lethality of their current heat treatment processes.

1. Introduction

The microbiological stability of shelf-stable fruit juices is based on the combination of their low pH values (usually ≤3.8) with heat treatments designed to inactivate the most heat resistant microorganisms found. Throughout the decades, several microorganisms have been used as targets of fruit juice pasteurization processes, including yeasts, lactic acid bacteria, heat resistant molds and spore-forming bacteria (Tribst et al., 2009). However, since early 80's, fruit juice processors have been challenged by a bacterium showing remarkable heat and chemical resistances, ability to grow under acidic conditions and, consequently, to spoil shelf-stable fruit juices (Silva and Gibbs, 2001; Friedrich et al., 2009; Spinelli et al., 2009, 2010). This bacterium was characterized by the presence of ω-alicyclic fatty acids as major lipid components on the cellular membrane, which together with 16S rRNA sequencing analyses led to the proposal for creation of a new genus, *Alicyclobacillus* (Wisotzkey et al., 1992). Currently, it is known that members of the *Alicyclobacillus* genus are surprisingly diverse and not all species have been described as containing these characteristic fatty acids (Glaeser et al., 2013). Presently, more than 20 species have been reported to belong to *Alicyclobacillus* genus (Smit et al., 2011; Glaeser et al., 2013), while spoilage potential of fruit juices has been restricted to few species such as *Alicyclobacillus acidoterrestris*, *Alicyclobacillus acidiphillus*, *Alicyclobacillus pomorum*, *Alicyclobacillus herbarius*, *Alicyclobacillus hesperidum*, *Alicyclobacillus acidocaldarius* and *Alicyclobacillus cycloheptanicus* (Cerny et al., 1984; Matsubara et al., 2002; Goto et al., 2003; AIJN, 2007; Smit et al., 2011). The spoilage potential of *Alicyclobacillus* species relies on their ability to produce off-flavor compounds such as 2-methoxyphenol (guaiacol), 2,6-dibromophenol, 2,6-dichlorophenol and 2-methyltetrahydrothiophene-3-one (Siegmund and Pöllinger-Zierler, 2006; Lottici et al., 2006; Siegmund and Pöllinger-Zierler, 2007; Concina et al., 2010).

Because of its spoilage potential, several reports are found on the incidence of *Alicyclobacillus* in fruit and vegetable beverages

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(Siegmund and Pöllinger-Zierler, 2006; Durak et al., 2010; Steyn et al., 2011; Walls and Chuyate, 2000; Groenewald et al., 2009; McKnight et al., 2010; Danyluk et al., 2011; Oteiza et al., 2011). Also, as a major target for fruit juice pasteurization (Tribst et al., 2009), numerous studies are found that report thermal inactivation parameters of *Alicyclobacillus*, *i.e.*, the *D* value (time at a determined temperature required to cause one-log cycle decrease in the population of a target bacterium) and the *z* value (temperature increase required to result in one-log cycle decrease of *D*-value) (Splittstoesser et al., 1994; Komitopoulou et al., 1999; Bahçeci and Acar, 2007; Walls, 1997; Silva et al., 1999; Maldonado et al., 2008; de Carvalho et al., 2008; López et al., 2011; Alberice et al., 2012, Peña et al., 2009; McKnight et al., 2010). As known, *D*- and *z*-values of *Alicyclobacillus* are affected by the particular conditions or *study characteristics* (protocols, fruit and variety, °Brix, pH, temperature, heating medium, culture medium, inactivation method, strains, etc.) under which they were obtained. Therefore, variability in *D*- and *z*-values among primary studies is expected to occur, even among studies investigating the same type of fruit beverage. Nonetheless, by means of *a posteriori* analysis and identification – from each of the primary studies – of the sources of variability impacting on the thermal inactivation parameters of *Alicyclobacillus*, it may be possible to explain, to some extent, the differences found among the study outcomes.

To this respect, meta-analysis, defined as a *"statistical analysis of a collection of analytic results for the purpose of integrating the findings from a large amount of primary studies"* (DerSimonian and Laird, 1986), allows (i) the explanation of the divergences in the study outcomes by the codification of study characteristics (i.e., moderating variables related to research design features, data collection procedures, type of samples, etc.) aiming to reduce the between-study heterogeneity or variability (Gonzales-Barron et al., 2013); and (ii) the accurate estimation of the overall outcome measure, with increased statistical power than using only a single study (Sutton et al., 2001). Despite the capabilities of meta-analysis, already long recognized in medicine and clinical studies, the application of this body of compiling statistical techniques in food safety and microbiology issues is recent (Gonzales-Barron et al., 2008; Gonzales-Barron and Butler, 2011; Den Besten and Zwietering, 2012; Gonzales-Barron et al., 2013). Thus, the first objective of this study is to compile all publicly-accessible published findings on the heat resistance of *A. acidoterrestris* in fruit beverages as affected by temperature and pH, and quantitatively summarize these outcomes by means of a meta-analytical model based on a Bigelow-type secondary predictive model. A second objective is to attempt to explain a proportion of the total between-study heterogeneity in the heat resistance parameters by incorporating available study characteristics to the basic model. The resulting meta-analysis model (i.e., a mixed-effects linear model based on the Bigelow equation) should be effective in estimating the thermal inactivation parameters, *D*- and *z*-values, for the various types of beverage considered.

2. Methodology

2.1. Data collection

Literature identification was conducted using electronic search through Google with key terms, both in English and in Portuguese, including: "*Alicyclobacillus*", "ATSB", "Acidothermophilic sporeforming bacteria", "heat resistance", "*D*-value", "thermal resistance", "inactivation", "fruit juice", "juice", "beverages". Also, literature for inclusion in the study was identified from bibliographic databases such as Pubmed, Science Direct and Scopus, using the same keywords. Data included studies electronically available in scientific journals and electronically from 1980 to 2014. A total of 55 studies on inactivation of *Alicyclobacillus* spores in fruit beverages were retrieved, however, these included also reports using high pressure processing, ultrasound, pulsed electric field and pulsed light. Nonetheless, for inclusion in the meta-analysis, only conventional heat-related studies were considered, which originated from peer-reviewed scientific papers. A second criterion used in the screening was the need for the primary study to model first-order reaction kinetics; said otherwise, studies reporting on inactivation of *Alicyclobacillus* in fruit beverages with no *D*-values were excluded from the meta-analysis. Additionally, for a primary study to be included in the meta-analysis, it had to report more than two *D*-values, measured either at different inactivation temperatures or at different beverage pH. The statistical reason for this was that, for the meta-analytical mixed-effects linear model explained in Section 2.2, the standard error about the z_T or z_{pH} value (inverse of the slope between log *D* and temperature or pH, respectively) of a particular experiment could be only measured with more than two points along a fitted straight line. This restriction caused the results from four primary studies to be omitted for the analysis: Yamazaki et al. (1997) who reported two *D*-values for orange juice; Baumgart (1999) with only one *D*-value for orange juice; Vieira et al. (2002) reporting one D-value for cupuaçu concentrate; and Baysal and Icier (2010) who reported only two *D*-values for orange juice. Thus, 11 primary studies were selected and considered appropriate for the meta-analysis model, providing a total of 142 *D*-values obtained at different inactivation temperatures and pH values (Table 1).

2.2. Description of the data set

Apart from the *D*-values, the corresponding beverage pH and the temperatures at which the isothermal experiments were conducted, additional information was also extracted from the primary studies. It is known that the content of soluble solids or °Brix of the beverage is an important physicochemical parameter affecting the heat resistance of *Alicyclobacillus* (Splittstoesser et al., 1998). However, as such information was not available for every primary study, a categorical variable "type of beverage" was created to assign fruit beverages either to a single strength juice or to a concentrate class. It was defined that *D*-values obtained from beverages of either Brix above 18°, or *concentrates* and *nectars* (stated as such in the primary studies yet with no indication of the level of soluble solids) were assigned to the "concentrates" category. Single strength juices presented an average concentration of soluble solids of 10.2% (ranging from 5.3 to 13.0%) while fruit concentrates presented an average concentration of 48.0% (ranging from 18.0 to 68.0%).

Another study characteristic to codify (or to disaggregate) was the fruit. *D*-values were assigned to ten different fruit classes: apple, berry, cupuaçu, grape, grapefruit, lemon, mango, orange, passion fruit and tangerine. A special class named as "model" (Table 1) was created within the moderating variable fruit to encompass the results from López et al. (2011) and Bahçeci and Acar (2007), who employed citrate phosphate McIlvaine buffer to estimate the heat resistance of *Alicyclobacillus* at different pH values. This buffer is an acidic solution that has been proposed to model thermal process and heat transfer studies in fruit products.

The third moderator variable was "clarification" to indicate whether or not fruit beverages underwent the normal clarification process followed by filtration to separate the particles in suspension in the beverage. This was a coded variable taking the value of 0 for non-clarified beverages and the value of 1 for clarified beverages. For the special case of the *model* category within the fruit moderating variable, the "clarified" class was assigned because of the low viscosity and the absence of particles in suspension in a buffer (Table 1). On the other hand, the study of de Carvalho et al. (2008), which focused on mango concentrate, did not specify

Table 1
Meta-analytical data of *D*-values of *Alicyclobacillus acidoterrestris* in beverages at different temperature and pH, with extracted study characteristics of fruit, type (single strength or concentrate), clarification (0 = no, 1 = yes), bacteriocins (0 = no, 1 = yes) and sample size *N* used to estimate a single *D*-value.

Fruit	Type	Clarification	Bacteriocins	pH	T (°C)	*D* (min)	*N*	Source
Apple	Single strength	0	0	3.50	85	56.0	20	Splittstoesser et al. (1994)
		0	0	3.50	90	23.0	20	
		0	0	3.50	95	2.80	20	
		1	0	3.51	80	41.2	18	Komitopoulou et al. (1999)
		1	0	3.51	90	7.38	22	
		1	0	3.51	95	2.30	22	
		1	0	3.68	90	11.1	25	Bahçeci and Acar (2007)
		1	0	3.68	93	4.20	25	
		1	0	3.68	96	2.10	25	
		1	0	3.68	100	0.70	25	
		1	1	3.51	80	23.8	18	Komitopoulou et al. (1999)
		1	1	3.51	90	4.56	22	
		1	1	3.51	95	1.95	22	
	Concentrate	1	0	2.97	90	14.4	25	Bahçeci and Acar (2007)
		1	0	2.97	93	6.70	25	
		1	0	2.97	96	3.30	25	
		1	0	2.97	100	1.20	25	
		1	0	2.95	90	14.1	25	Bahçeci and Acar (2007)
		1	0	2.95	93	6.40	25	
		1	0	2.95	96	3.10	25	
		1	0	2.95	100	1.00	25	
Berry	Single strength	1	0	3.50	88	11.0	20	Walls (1997)
		1	0	3.50	91	3.80	20	
		1	0	3.50	95	1.00	20	
Cupuaçu	Single strength	1	0	3.60	85	17.5	20	Silva et al. (1999)
		1	0	3.60	91	5.35	20	
		1	0	3.60	95	2.82	20	
		1	0	3.60	97	0.57	20	
Grape	Single strength	1	0	3.30	85	57.0	20	Splittstoesser et al. (1994)
		1	0	3.30	90	16.0	20	
		1	0	3.30	95	2.40	20	
Grapefruit	Single strength	1	0	3.42	80	37.8	18	Komitopoulou et al. (1999)
		1	0	3.42	90	5.95	22	
		1	0	3.42	95	1.85	22	
		1	0	3.00	80	31.85	18	Komitopoulou et al. (1999)
		1	0	3.00	90	5.69	22	
		1	0	3.00	95	1.49	22	
		1	0	4.00	80	52.35	18	Komitopoulou et al. (1999)
		1	0	4.00	90	9.44	22	
		1	0	4.00	95	1.73	22	
Lemon	Single strength	0	0	2.45	82	16.72	20	Maldonado et al. (2008)
		0	0	2.45	86	11.32	20	
		0	0	2.45	92	10.58	20	
		0	0	2.45	95	9.98	20	
		0	0	2.45	82	17.82	20	
		0	0	2.45	95	9.44	20	
		1	0	3.50	82	11.23	20	Maldonado et al. (2008)
		1	0	3.50	86	10.54	20	
		1	0	3.50	92	9.47	20	
		1	0	3.50	95	8.55	20	
		1	0	3.50	82	13.21	20	
		1	0	3.50	95	9.38	20	
	Concentrate	0	0	2.28	82	15.50	20	Maldonado et al. (2008)
		0	0	2.28	86	14.54	20	
		0	0	2.28	92	8.81	20	
		0	0	2.28	95	8.55	20	
		0	0	2.80	82	50.50	20	Maldonado et al. (2008)
		0	0	2.80	86	39.30	20	
		0	0	2.80	92	31.67	20	
		0	0	2.80	95	22.03	20	
		0	0	3.50	82	95.15	20	Maldonado et al. (2008)
		0	0	3.50	86	59.50	20	
		0	0	3.50	92	38.00	20	
		0	0	3.50	95	17.22	20	
		0	0	4.00	82	85.29	20	Maldonado et al. (2008)
		0	0	4.00	86	58.15	20	
		0	0	4.00	92	27.48	20	
		0	0	4.00	95	23.33	20	
		0	0	2.45	82	15.50	20	Maldonado et al. (2008)
		0	0	2.45	86	14.54	20	

(*continued on next page*)

Table 1 (*continued*)

Fruit	Type	Clarification	Bacteriocins	pH	T (°C)	*D* (min)	*N*	Source
		0	0	2.45	92	8.81	20	
		0	0	2.45	95	8.56	20	
		1	0	2.28	82	17.36	20	Maldonado et al. (2008)
		1	0	2.28	86	18.06	20	
		1	0	2.28	92	7.60	20	
		1	0	2.28	95	6.20	20	
		1	0	2.80	82	25.81	20	Maldonado et al. (2008)
		1	0	2.80	86	22.01	20	
		1	0	2.80	92	15.35	20	
		1	0	2.80	95	11.3	20	
		1	0	3.50	82	68.9	20	Maldonado et al. (2008)
		1	0	3.50	86	33.7	20	
		1	0	3.50	92	16.8	20	
		1	0	3.50	95	12.6	20	
		1	0	4.00	82	35.2	20	Maldonado et al. (2008)
		1	0	4.00	86	23.2	20	
		1	0	4.00	92	21.9	20	
		1	0	4.00	95	9.72	20	
		1	0	3.50	82	18.1	20	Maldonado et al. (2008)
		1	0	3.50	86	17.4	20	
		1	0	3.50	92	7.60	20	
		1	0	3.50	95	6.20	20	
Mango	Concentrate	0	0	4.00	80	40.0	15	de Carvalho et al. (2008)
		0	0	4.00	85	25.0	15	
		0	0	4.00	90	11.7	15	
		0	0	4.00	95	8.33	15	
		0	1	4.00	80	9.20	15	de Carvalho et al. (2008)
		0	1	4.00	85	5.00	15	
		0	1	4.00	90	1.16	15	
		0	1	4.00	95	0.36	15	
Model	Single strength	1	0	3.00	90	6.00	25	Bahçeci and Acar (2007)
		1	0	3.00	93	2.80	25	
		1	0	3.00	96	1.10	25	
		1	0	3.00	100	0.40	25	
		1	0	3.50	90	6.50	25	Bahçeci and Acar (2007)
		1	0	3.50	93	3.20	25	
		1	0	3.50	96	1.30	25	
		1	0	3.50	100	0.40	25	
		1	0	4.00	90	7.30	25	Bahçeci and Acar (2007)
		1	0	4.00	93	3.80	25	
		1	0	4.00	96	1.70	25	
		1	0	4.00	100	0.50	25	
		1	0	3.50	90	6.00	18	López et al. (2011)
		1	0	3.50	95	2.20	18	
		1	0	3.50	100	0.83	18	
		1	0	3.50	105	0.34	18	
Orange	Single strength	1	0	3.90	80	54.3	18	Komitopoulou et al. (1999)
		1	0	3.90	90	10.3	22	
		1	0	3.90	95	3.59	22	
		1	0	3.57	80	16.3	15	Alberice et al. (2012)
		1	0	3.57	87	12.5	15	
		1	0	3.57	95	10.8	12	
		1	0	3.57	99	1.38	12	
	Concentrate	0	0	3.68	92	25.6	10	Peña et al. (2009)
		0	0	3.68	95	12.9	10	
		0	0	3.68	98	6.16	10	
		0	0	3.68	102	2.01	10	
		0	1	3.68	95	11.4	10	Peña et al. (2009)
		0	1	3.68	98	5.55	10	
		0	1	3.68	102	1.83	10	
		1	0	2.95	80	18.4	15	Alberice et al. (2012)
		1	0	2.95	87	13.4	15	
		1	0	2.95	95	10.6	12	
		1	0	2.95	99	1.67	12	
Passion fruit	Single strength	1	0	3.50	87	20.9	12	McKnight et al. (2010)
		1	0	3.50	90	5.12	12	
		1	0	3.50	95	1.62	12	
Tangerine	Single strength	0	0	3.50	90	15.0	18	López et al. (2011)
		0	0	3.50	95	6.20	18	
		0	0	3.50	100	2.10	18	
		0	0	3.50	105	0.63	18	

whether the concentrate was clarified or not. However, as the main objective of such a study was to assess the effect of bovicin on the resistance of *Alicyclobacilus* in mango pulp, a logical conclusion was that the mango pulp, which was two-fold diluted for their experiments (i.e., concentrate), was not clarified.

The fourth study characteristic was "presence of bacteriocins", which was conceived because two of the primary studies investigated the effect of nisin (Komitopoulou et al., 1999; Peña et al., 2009) on the thermal resistance of *Alicyclobacillus*; and one study the effect of bovicin HC5 — a bacteriocin from *Streptococcus bovis* (de Carvalho et al., 2008). Thus, this categorical variable was coded to take up the value of 0 for absence of bacteriocins and the value of 1 for added bacteriocins. While de Carvalho et al. (2008) employed a concentration of bovicin HC5 of 80 IU/ml in mango concentrate, Komitopoulou et al. (1999) and Peña et al. (2009) assessed both a concentration of 50 IU/ml in apple and orange juice, respectively.

A summary of the input data for the meta-analysis study is presented in Table 1. It should be noticed that such meta-analytical data is highly sparse, meaning that for some fruits less data are available. For instance, for apple, lemon and orange, data for both types of beverages — juice and concentrate — were found, and additionally for clarified and non-clarified beverages, while for other fruits such as grape and passion fruit, data were limited to clarified juices only. This has some implications in the design of the meta-analysis mixed-effects model, as explained in Section 2.3.

2.3. Meta-analytical model

To describe the combined effect of temperature and pH on the heat resistance of *Alicyclobacillus* in fruit beverages, the Bigelow-type linear model was selected (Mafart and Leguerinel, 1998):

$$\log D = \log D^* - \left(\frac{1}{z_T}\right)(T - T^*) - \left(\frac{1}{z_{pH}}\right)(\mathrm{pH} - \mathrm{pH}^*) \quad (1)$$

where D is time at a constant temperature T and at the pH of the food matrix required to cause one-log cycle decrease in the population of a target bacterium; T^* is the reference temperature (set at 95 °C, which is a common temperature for fruit juice pasteurization); pH* is the reference pH (chosen to be 3.5 to correspond to a common pH of fruit beverages); z_T is the conventional thermal z-value; z_{pH} is the distance of pH from pH* which leads to a ten-fold reduction of the decimal reduction time; and D^* is the decimal reduction time at T^* and pH*.

The Bigelow secondary predictive model was used to interpret the combined results of the primary studies. As the meta-analytical data obtained also contain a number of moderating variables or coded study characteristics (for example, fruit, type of beverage, addition of bacteriocin and application of clarification), the Bigelow model was transformed into a linear mixed-effects model in order to assess whether each of the moderating variables has any effect on D^* and/or z_T and z_{pH}. Hence, the three parameters of Equation (1) were modelled as.

$$\log D^*_{ijlm} = (\beta_0 + \beta_{1i} + \beta_{2j}) + u_{lm} = \log D^*_{\mathrm{mean}\ ij} + u_{lm} \quad (2)$$

$$\frac{1}{z_{T\,ilm}} = (\gamma_1 + \gamma_{2i} + v_{lm}) \quad (3)$$

$$\frac{1}{z_{\mathrm{pH}_k}} = (\delta_1 + \delta_{2k}) \quad (4)$$

Where: β_0 is an intercept, β_1 is the fixed effect of the type of beverage i (coded as 0 for single strength juice and 1 for concentrates), β_2 is the fixed effect of the clarification stage j (coded as 0 for no clarification and 1 for regular clarification). A fixed effect of the addition of bacteriocin on log D^* was not considered as it turned out to be non-significant. The value of $D^*_{\mathrm{mean}\ ij}$ represents the average decimal reduction time at the reference T^* and pH* applicable to the entire population of fruits, yet it is an intercept allowed to take up different independent values due to the variability in the fruit/primary study combination (viz. interaction). Because of the sparse nature of the data structure, whereby in most cases one primary study reported results for only one fruit (Table 1), for the analysis it was not feasible either to separate the between-fruit variability from the between-study variability or to build a nested covariance of primary studies within fruit or fruits within primary study. To overcome this problem and still be able to account for the evident variability due to the different fruits (l) and primary studies (m), both variables had to be merged into an interaction variable (lm) providing sixteen levels to be used as the subject of variation of the random effects placed in Equation (2). These intercept random effects u_{lm} are assumed to have a normal distribution with mean zero and variance s^2_u.

The coefficient γ_1 is the mean effect of a 1°C-increment in temperature (T–T^*) for the entire population of fruit beverages; yet, the coefficient for the temperature difference slope is affected by the type of beverage i and by the specific combination of fruit (l) and primary study (m). Neither clarification j nor bacteriocin k was included as a predictor of the temperature difference slope because they were not statistically significant. γ_2 is the fixed effect of the interaction term between the type of beverage i and the temperature slope. Since preliminary analysis of the meta-analytical data had shown that the temperature slopes for single strength juice tended to be steeper than those for concentrates, this variability was accounted for. As done for the intercept random effects, the interaction between fruit and primary study (lm) was assumed to be the subject of variation of the random effects v_{il}. The random effects v_{il} added to the slope $\gamma_1 + \gamma_2$ model the shifts in the temperature effect for each of the primary study × fruit existing in the data set. These slope random effects are assumed to follow a normal distribution with mean zero and variance s^2_v. Placing a fixed effect on the type of beverage and random effects for fruits (interacting with the primary studies) in Equation (3) enables the model to compute the z_T values for all the combinations of fruit and type of beverage, even beyond the combinations existing in the original meta-analytical data.

The coefficient δ_1 represents the effect of the increment in the pH difference (pH–pH*), and δ_2 the coefficient of the interaction term between addition/non-addition of a bacteriocin (k) and the pH slope. This interaction allows for a change in the pH difference slope when a bacteriocin is added to the beverage. Fixed effects of the type of beverage i and the application of clarification j were not included in Equation (3) for being non-significant. Random variations in the pH slope due to beverage type and fruit were not modelled in Equation (3) as they turned out to be non-significant. The variances of the random effects placed on the intercept and temperature slope, s^2_u and s^2_v, were assumed to be correlated with a covariance s^2_{uv}. As all those variance and covariance terms can be thought of as realisations of a *primary study*, the presence of heterogeneity among primary studies can be assessed by the Wald's test of significance of each of the variance, s^2_u and s^2_v, and covariance s^2_{uv} parameters. Hence, if those terms were statistically significant, the between-study variability τ^2 can be approximated by $s^2_u + s^2_v + s^2_{uv}$, and the I^2 statistics or intra-class correlation, estimating the proportion of between-study variability from the total variance, can be approached as $(s^2_u + s^2_v + s^2_{uv})/(s^2_u + s^2_v + s^2_{uv} + s^2)$, where s^2 is the variance of the normally-distributed residual random errors ε_{ijklm}.

Thus, putting together Equations (2)–(4), the linear mixed-effects model adjusted to the meta-analytical data was.

$$\log D_{ijklm} = (\beta_0 + \beta_{1i} + \beta_{2j}) + u_{lm} - (\gamma_1 + \gamma_{2i} + v_{lm})(T - T^*) - (\delta_1 + \delta_{2k})(\mathrm{pH} - \mathrm{pH}^*) + \varepsilon_{ijklm} \quad (5)$$

Notice that the values of log D^*, z_T and z_{pH} can be estimated from the model's fitted parameters using Equations (2)–(4), respectively. In building the meta-analysis mixed model, all the interaction terms between the categorical moderating variables, and with pH and temperature were evaluated. Because of data sparseness, only interactions of two terms were considered. However, only two interaction terms were found to be statistically significant (i.e., slope of temperature difference with type of beverage and slope of pH with presence of bacteriocins), which were retained in the model. Similarly, a series of combinations of random effects attempting to extract the variability between fruits and the variability between primary studies, both separately and as interactions, were placed in Equations (2)–(4), and their results compared one-to-one by a log-likelihood ratio test and the Bayesian Information Criterion (BIC). The model presented in Equation (5) was the most parsimonious (i.e., least parameters with the best goodness-of-fit), and yet, with a fully interpretable arrangement. Since primary studies are expected to differ from each other in the reliability of estimating the true heat resistance parameters of *A. acidoterrestris* in fruit beverages, for instance, due to differences in study sizes, a *weighted* linear mixed model was preferred, with weights representing the precision in estimating the population lethality parameters. Because not all primary studies reported the standard error of the *D*-value, the precision was defined as some measure proportional to the sample size N used in the bacterial kinetics experiments to calculate a single *D*-value. Hence, the weight – level of confidence on each *D* measure – was given by the sample size. Table 1 also compiles the sample size used to determine each of the *D*-values, which was calculated as the number of sample units analysed multiplied by the number of points in time where samples were taken to measure the concentration of *Alicyclobacillus*. Once the model was fitted, the normality of residuals was assessed and the studentised residuals examined for identifying spurious data points lower than −3.0 and higher than 3.0. The weighted mixed-effects linear model was fitted in R version 2.14.2 (R Development Core Team) using the 'nlme' package (Pinheiro et al., 2013).

3. Results and discussion

The management of microbial spoilage of fruit beverages requires the ability to predict the thermal resistance of the spores of *A. acidoterrestris*. During this systematic review, it was realized that there are in the literature numerous studies reporting useful data on the thermal death kinetics of this spoilage microorganism, which, in principle, could be applied for the determination and optimisation of the process variables for heat treatment. However, the large number and variety of data, and principally, the different estimates of the thermal inactivation parameters among studies, make further developments difficult. For instance, the study of Komitopoulou et al. (1999) reported a z_T-value of 12.9 °C for orange juice at a pH of 3.9, while Yamazaki et al. (1997) found a lower z_T-value of 9.5 °C for orange juice at a similar pH of 3.7. Similarly, for apple juice at a pH of 3.5, Komitopoulou et al. (1999) and Splittstoesser et al. (1994) found dissimilar z_T-values of 12.2 °C and 7.7 °C, respectively. The degree of discrepancies in the relationship between *D*-value and temperature observed in the input data set can be visually assessed in Fig. 1. In such a *Figure*, the same markers depict a sub-group of observations from a given set of heat inactivation isothermal experiments conducted to determine a z_T value at fixed conditions; said otherwise, a sub-group is formed by the paired observations (*D*-value, temperature) extracted for a given fruit, type of beverage, clarification, bacteriocin and pH value. From Table 1, it can be deduced that there were 37 sub-groups. Fig. 1 shows that the *D*-values from the 37 sub-groups were all consistent as they decrease with increasing temperatures, yet it also hinted that, in designing a meta-analytical linear model, some allowance had to be made in relation to the variability of the intercepts and slopes (inverse of z_T) by incorporating random effects. In a multilevel meta-analysis, as is the case here, one usually starts assessing the null random-effects model. In our case, the null random-effects model is the simple Bigelow model (Equation (1)) with random effects placed on the intercept and the temperature difference slope. Such a model produced a value of heterogeneity τ^2 of 0.072 while the variance of the residuals was 0.094 (results not shown). Thus, the intra-class correlation can be estimated ($I^2 = 0.072/(0.072 + 0.094) = 0.44$) at 44%. This value, being higher than the rule of thumb of 25% (Hunter and Schmidt, 1990), underscored the presence of significant heterogeneity; and, consequently, confirmed that some study characteristics had to be coded in an attempt to explain, understand and reduce such variability.

When the null random-effect model (basic Bigelow) was extended to a multilevel model (mixed-effects linear model comprising study characteristics or moderating variables; Equation (5)), the variance of the residuals reduced to 0.038, and the heterogeneity τ^2 reduced to 0.044 (Table 2). This indicated that approximately 40% ((0.072−0.044)/0.072 = 0.389) of the total amount of heterogeneity due to primary studies and fruits could be explained by the categorical variables type of beverage, clarification and presence of bacteriocins. Because the residual heterogeneity τ^2 of 0.044 is still significant (Table 2), it can be concluded that there may be other study characteristics, not coded in the present meta-analysis, that are likely to be also noteworthy. As Hox and De Leeuw (2003) pointed out, it is highly unlikely that the available study-level variables could cover all the artefacts causing variation between study outcomes. This occurs because the information given in research reports and articles is not enough to cover all the study characteristics; and in fact this was attested during the conduction of the present meta-analysis. For instance, while the specific strain inoculated in the essay and the method used to measure heat resistance may explain some of the between-study variability observed among the measured *D*-values, they could not be considered in the model since not all primary studies reported such information.

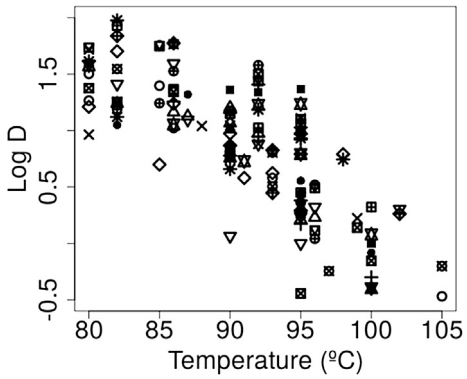

Fig. 1. Scatter plot of the available meta-analytical data of log *D* and temperature for the 37 sub-groups of isothermal experiments to determine a *z*-value.

Table 2
Parameter estimates of the Bigelow-type meta-analysis mixed-effects linear model predicting the log *D*-value of *Alicyclobacillus acidoterrestris* in fruit beverages as a function of temperature, pH and moderating variables.

Parameters	Mean	Standard error	Pr > |t|, Z	AIC/BIC
Predictors of log D^*				
β_0 (intercept)	0.396	0.056	<0.0001	−80.0/−50.0
β_1 (type)	−0.115	0.048	0.018	
β_2 (clarification)	−0.261	0.037	<0.0001	
Predictors of ($1/z_T$)				
γ_1 (temperature)	−0.089	0.008	<0.0001	
γ_2 (temperature × type)	0.014	0.006	0.025	
Predictors of ($1/z_{pH}$)				
δ_1 (pH)	1.707	0.207	<0.0001	
δ_2 (pH × bacteriocin)	−1.881	0.206	<0.0001	
Variances				
s^2_u (log D^*_{mean})	0.0389	0.0162	0.008	τ^2~0.044
s^2_v (temperature)	0.0010	0.0004	0.012	I^2~53.9%
s^2_{uv} (covariance)	0.0045	0.0023	0.050	
s^2 (residual)	0.0380	0.0053	<0.0001	

Table 3
Estimates of log D^* (log *D*-value at 95 °C and pH 3.5) for different combinations of fruits and the moderating variables, type of beverage and with/without clarification process.

Parameter	Mean	Standard error	95% CI
Overall mean	0.584	0.055	[0.474–0.694]
Single strength juice	0.526	0.056	[0.414–0.638]
Clarified	0.396	0.056	[0.285–0.507]
Non-clarified	0.656	0.063	[0.532–0.781]
Concentrate	0.642	0.064	[0.514–0.769]
Clarified	0.511	0.068	[0.376–0.645]
Non-clarified	0.772	0.066	[0.641–0.903]
Apple single strength juice	0.470^{c}	0.047	[0.377–0.565]
Berry single strength juice	0.252^{a}	0.108	[0.036–0.467]
Cupuaçu single strength juice	0.355^{b}	0.078	[0.200–0.510]
Grape single strength juice	0.577^{d}	0.109	[0.361–0.793]
Grapefruit single strength juice	0.437^{bc}	0.064	[0.310–0.564]
Lemon single strength juice	1.007^{f}	0.048	[0.912–1.103]
Mango single strength juice	0.425^{b}	0.102	[0.223–0.628]
Orange single strength juice	0.695^{e}	0.054	[0.587–0.803]
Passion fruit single strength juice	0.401^{b}	0.130	[0.142–0.660]
Tangerine single strength juice	0.594^{d}	0.067	[0.462–0.727]
Apple concentrate	0.586^{c}	0.053	[0.470–0.702]
Berry concentrate	0.367^{a}	0.115	[0.138–0.596]
Cupuaçu concentrate	0.470^{b}	0.088	[0.295–0.646]
Grape concentrate	0.693^{d}	0.115	[0.463–0.922]
Grapefruit concentrate	0.552^{bc}	0.076	[0.401–0.703]
Lemon concentrate	1.122^{f}	0.036	[1.050–1.195]
Mango concentrate	0.541^{b}	0.096	[0.348–0.732]
Orange concentrate	0.810^{e}	0.053	[0.705–0.915]
Passion fruit concentrate	0.517^{b}	0.136	[0.247–0.787]
Tangerine concentrate	0.709^{d}	0.084	[0.544–0.876]
Apple single strength juice			
Clarified	0.340	0.049	[0.243–0.438]
Non-clarified	0.601	0.052	[0.497–0.705]
Apple concentrate			
Clarified	0.456	0.064	[0.328–0.583]
Non-clarified	0.716	0.058	[0.601–0.832]
Mango single strength juice			
Clarified	0.295	0.104	[0.088–0.502]
Non-clarified	0.555	0.103	[0.350–0.761]
Mango concentrate			
Clarified	0.410	0.101	[0.209–0.612]
Non-clarified	0.671	0.095	[0.482–0.860]
Orange single strength juice			
Clarified	0.565	0.053	[0.458–0.670]
Non-clarified	0.825	0.062	[0.703–0.947]
Orange concentrate			
Clarified	0.680	0.057	[0.567–0.793]
Non-clarified	0.940	0.055	[0.830–1.051]

*Different superscript letters denote statistical differences across fruits separately for single strength juices and for concentrates.

As expected, the inactivation temperature affected ($p < 0.0001$) the resistance of *Alicyclobacillus* (Table 2). In comparison with the predominant effect of temperature (*F*-value = 100.7), the influence of pH on the heat resistance of *Alicyclobacillus* was weaker (*F*-value = 32.5), as suggested by the more disperse scatter plot between log *D* and beverage pH (not shown). Nonetheless, the meta-analysis model was still able to detect the significance of this physicochemical property (Table 2). In an earlier study, Pontius et al. (1998) detected as well a significant effect of pH, although they showed that it becomes more notorious only at lower inactivation temperatures. In this work, as the summarised data comprised a narrow range of pH from 2.8 to 4.0, it is natural that the effect of higher temperatures (from 80 °C) surpasses the effect of the matrix acidity. Although the mechanisms of resistance to pasteurisation of *Alicyclobacillus* are still unclear, the thermal resistance of other bacterial spores is influenced by several environmental factors such as pH, water activity and menstruum composition (Baysal and Icier, 2010). However, the most significant parameter in the inactivation of microorganisms is the thermal effect itself, regardless of the type of thermal treatment.

The heat sensitivity of *Alicyclobacillus* was shown to be significantly different between single strength juices and concentrates (i.e., see variable type in Table 2). Independently of the kind of fruit, the concentrates had on average log D^* values higher than juices by 0.115 units. This finding was in agreement with Alberice et al. (2012), who found that the *D*-values in all temperatures assayed were slightly higher in concentrated juice than in reconstituted juice. An explanation of the fact that the inactivation rate of *Alicyclobacillus* is higher in single strength juices than in concentrates can be found in Gombas (1983), who sustained that an apparent increase in spore heat resistance is achieved when it is balanced in low water activity or dissolved in a solution of high osmotic potential. High sugar concentrations like sucrose exert a similar osmotic pressure that exists in the spore cortex. Thus, protoplast dehydration is induced mechanically and osmotically by pressure, and this dehydration mechanism present in the spores is probably responsible for heat resistance.

In our meta-analysis study, the type of beverage was not only responsible for causing a shift in the intercept (log D^*) of the relationship between log *D* and temperature but also for causing a shift in the slope. Notice that the interaction term temperature × type is significant ($p < 0.05$; Table 2), therefore bringing about differences in z_T values for juices and concentrates (Table 4). The estimate of 0.014 for temperature × type (Table 2) indicates that, in single strength juices, the slope between log *D* and temperature is higher (steeper) than in concentrates by 0.014 units. In other words, the same increase in the pasteurisation temperature for concentrates will have a lower effect on the heat resistance of *Alicyclobacillus* than for juices. This is, as a consequence, reflected in the z_T values estimated by the meta-analysis model (Table 4), which in all cases are higher in concentrates than in single strength juices.

It was also demonstrated that *Alicyclobacillus* possesses less thermal resistance in clarified beverages than in non-clarified beverages. In the meta-analysis model, the variable clarification had an effect ($p < 0.0001$) on the *D*-values as a single term (Table 2) but not in interactions either with temperature or with pH (results not shown). Hence, clarification only affects the estimation of log D^*, meaning that, in the relationships between log *D* and temperature or log *D* and pH, the process of clarification will only cause a downward shift in the straight line, and will not affect either the temperature slope or the pH slope; hence, will not affect the z_T or z_{pH} values. On average, the model estimated that a non-clarified

Table 4
Estimates of z_T (°C) and z_{pH} obtained by the meta-analytical secondary predictive model.

Parameter	Mean	Standard error	95% CI
z_T			
Single strength juices – all fruits	11.23	1.107	[9.034–13.42]
Concentrates – all fruits	13.35	1.744	[9.893–16.80]
Apple single strength juice	10.34[c]	0.728	[8.898–11.78]
Berry single strength juice	8.019[ab]	1.354	[5.339–10.70]
Cupuaçu single strength juice	9.261[b]	1.163	[6.958–11.56]
Grape single strength juice	7.957[a]	1.059	[5.860–10.06]
Grapefruit single strength juice	11.17[c]	0.907	[9.378–12.96]
Lemon single strength juice	15.86[e]	1.467	[12.95–18.76]
Mango single strength juice	17.39[e]	3.375	[10.71–24.08]
Orange single strength juice	12.48[d]	1.162	[10.17–14.78]
Passion fruit single strength juice	8.907[b]	1.789	[5.365–12.45]
Tangerine single strength juice	11.35[c]	1.382	[8.611–14.08]
Apple concentrate	12.19[c]	1.164	[9.886–14.50]
Berry concentrate	9.045[ab]	1.780	[5.520–12.57]
Cupuaçu concentrate	10.65[b]	1.682	[7.326–14.00]
Grape concentrate	8.967[a]	1.424	[6.147–11.78]
Grapefruit concentrate	13.27[c]	1.679	[9.945–16.59]
Lemon concentrate	20.43[e]	1.827	[16.81–24.05]
Mango concentrate	23.07[e]	5.054	[13.06–33.08]
Orange concentrate	15.15[d]	1.752	[11.68–18.62]
Passion fruit concentrate	10.19[b]	2.408	[5.422–14.96]
Tangerine concentrate	13.52[c]	2.269	[9.023–18.01]
z_{pH}			
Overall mean	1.305	0.180	[0.948–1.661]
With bacteriocin	0.586	0.071	[0.445–0.726]
Without bacteriocin	5.750	0.950	[3.869–7.631]

*Different superscript letters denote statistical differences across fruits separately for single strength juices and for concentrates.

beverage will exhibit an increase in the intercept or log D^* value by 0.26 units (Table 2). It may be hypothesised that the greater particles in suspension in a non-clarified juice slows down the heat transfer rate, retarding also the thermal inactivation of *Alicyclobacillus*. This is also affected by the method employed to assess microbial thermal resistance. For instance, the most common method of inoculating the microorganism in small closed vessels and immersing them in the heating medium, leads to the production of non-desirable heating lag times, which will accentuate the difference in D-values estimates between clarified and non-clarified beverages. On the contrary, methods whereby the inoculum is added to the sample only when it reaches the desired temperature will produce an insignificant thermal lag, leading to more accurate D-values, and probably smaller differences between clarified and non-clarified juices. The bias caused by the method used to determine microbial thermal resistance could not be assessed in the present meta-analysis as some primary studies failed to report the method in a clear way.

The meta-analysis also demonstrated that there is a significant effect of the addition of bacteriocins prior to heating on the thermal resistance of *Alicyclobacillus*, increasing the lethality of pasteurisation. Although the variable bacteriocin was not statistically significant when it entered the model as a single term (i.e., as a predictor of log D^*), it was highly significant as an interaction term with pH (Table 2). The negative estimate of pH × bacteriocin suggests that for a constant value of beverage pH, the addition of bacteriocins (either nisin or bovicin in the doses studied in their respective primary studies) will increase the thermal sensitivity of *Alicyclobacillus* (i.e., lower log D). On the other hand, the fact that there is an interaction between pH and the presence of bacteriocins implies that the effect of a bacteriocin on the thermal sensitivity of *Alicyclobacillus* becomes more evident at higher pH. This is, a greater bactericide effect is revealed when a bacteriocin is added to a less acidic beverage in comparison to a highly acidic beverage. This may stem from both of the following reasons: Firstly, in a highly acidic matrix, the effect of the low pH itself on *Alicyclobacillus* lethality may mask the effect of the bacteriocin, and hence, the effect of the latter becomes less evident. Secondly, there is a direct effect of pH on bacteriocin activity, which is higher at lower pH values (Davies et al., 1998; Houlihan et al., 2004). With this, the lower the pH of the matrix, the more active the bacteriocin becomes, and the more strongly *Alicyclobacillus* is inhibited, causing, at that lower pH, a greater increase in heat sensitivity in comparison to that when no bacteriocin was added.

As a consequence, the value of z_{pH} estimated for beverages with bacteriocins (0.586) was significantly lower (i.e., the spore heat resistance is highly affected by changes in pH) than the one for beverages without bacteriocins (5.750) (Table 4). The bacteriocins in doses between 50 and 80 IU/ml reduced by a factor of ten the z_{pH} value of *Alicyclobacillus*. In this meta-analysis study, the addition of bacteriocins did not play a role on the reduction of z_T as the interaction temperature × bacteriocin turned out to be non-significant. Yet, our model still confirmed that the bacteriocins, nisin and bovicin, were bactericidal against *Alicyclobacillus*, as the D-values – hence, the viable cell numbers – decreased in their presence. Although there is evidence that higher doses of bacteriocins have greater effect on increasing the lethality of *Alicyclobacillus* spores (Peña et al., 2009; Komitopoulou et al., 1999), this was not assessed in this meta-analysis study.

The variances s_u^2 and s_v^2 of the random effects placed on the model's intercept (log D^*) and temperature slope, respectively, were both significant (Table 2), confirming statistically the presence of heterogeneity that was initially observed in Fig. 1. As the subject of variation of the random effects was the interaction study × fruit, it can be conceived (i) that there is an infinite population (past, present and future) of primary studies reporting lethality data of *Alicyclobacillus* for a fruit beverage (ii) that there is an infinite population of fruits that can be subject of study; and (iii) that each of the studies associated to a fruit introduces inherent heterogeneity in the reported outcomes because of the differences in the methods for assessing microbial thermal resistance, in the composition of the beverage, in the bacteria strains inoculated, in the microbiological essay to quantify *Alicyclobacilus*, etc. As explained before, the fixed effects or coded study characteristics could explain 40% of such heterogeneity. Yet, there is a residual heterogeneity (τ^2~0.044; Table 2), which is still significant. The purpose of the random effects is therefore to absorb this unexplained heterogeneity.

Because “primary study” and “fruit” could not enter the meta-analysis model as separate subjects of random effects – since in the input data, in most cases, one primary study was associated to one fruit (Table 1) – consequently, the estimate of variability cannot be separated into that due to differences among primary studies and that due to differences among fruits. By entering primary study in interaction with fruit, both subjects of variability are acknowledged although they cannot be disaggregated. At most, it could be hypothesised that a primary study involves many more sources of variability in the estimates of bacterial heat resistance than the kind of fruit does; and therefore, that the between-study heterogeneity is much greater than the between-fruit heterogeneity. Based on this assumption, the between-study heterogeneity τ^2 was approximated by using the variances s^2_u, s^2_v and the covariance s^2_{uv} (Table 2).

Nevertheless, using such a model design, it is possible to provide estimates of log D^* and z_T for beverages (single strength juices or concentrates) of any of the ten fruits considered. This is possible by computing the random effects u_{lm} and v_{lm} (Equations (2) and (3), respectively) for a given fruit, and average them over the primary studies associated with such a fruit – in case that more than one

primary study was in interaction with that fruit. In this way, the log D^* and z_T-values were estimated for single strength juices and concentrates made of different fruits (Tables 3 and 4). A test of contrasts showed that there are statistical differences in the log D^* and z_T-values among the kinds of beverage. For instance, in terms of the D-value at 95 °C and at matrix pH of 3.5, *Alicyclobacillus* in berry juice presented a low heat resistance of 1.8 min (log $D^* = 0.252$ in Table 3), while in orange juice exhibited a higher thermal resistance with a D-value of 4.9 min (log $D^* = 0.695$ in Table 3). The growth and inactivation of *Alicyclobacillus* spores in commercial beverages depends, among other factors, on the compositional properties of food. For instance, in Splittstoesser et al. (1994), apple and tomato juice consistently supported growth, whereas grape juice at both pH 2.9 and 3.3 did not permit it. Different components present in fruits might increase the heat resistance of *Alicyclobacillus* spores, and this was clear for apple juice and apple nectar in Bahçeci and Acar (2007). Similar levels of heat resistance of *Alicyclobacillus* were found for tangerine juice (López et al., 2011) and orange juice (Conesa et al., 2009). Our meta-analysis study produced also relatively high log D^* values for tangerine and orange juice (Table 3). Nonetheless, because of the structure of our meta-analysis model, we cannot conclude that such significant differences in log D^* between, for instance, berry and orange juice (Table 3), can be entirely assigned to the composition of the fruits since it may as well be due to the heterogeneity among the primary studies that determined the D-values of *Alicyclobacillus* in berry and orange juice. Remember that the random effects had as subject the interaction primary study and fruit. Hence, some care should be taken in the interpretation of the statistical differences in the D-values and z-values estimates of the beverages across fruits listed in Tables 3 and 4. It is more prudent instead to interpret each of these estimates as *mean effect size* or overall average from all the meta-analysed literature sources. In fact, such summarisation of the research outcomes (i.e., available knowledge) increases the statistical confidence of the individual studies alone, and it is what constitutes one of the strengths of meta-analysis.

The mixed-effects linear model estimated a mean D^* value of 3.8 min with a 95% CI: 3.0–4.9 min (log $D^* = 0.584$; 95% CI: 0.474–0.694 in Table 3) to decrease one-log population of *Alicyclobacillus* in fruit beverages, on average (single strength juices or concentrates, clarified or non-clarified), at a temperature of 95 °C and a pH of 3.5. As this value is an estimate from a random-effects model, it can be generalised to the entire population of fruits and primary studies. More specifically, the mean D^* value estimate for single strength juices, whether clarified or not (3.3 min; 95% CI: 2.6–4.3 min), was lower ($p < 0.05$) than for the concentrates (4.4 min; 95% CI: 3.3–5.9 min). The mean D^* value for clarified juices (2.5 min; 95% CI: 1.9–3.2 min) was significantly lower than for non-clarified single strength juices (4.5 min; 95% CI: 3.4–6.0 min), and the same can be said for the clarified concentrates (3.2 min; 95% CI: 2.4–4.4 min) and the non-clarified concentrates (5.9 min; 95% CI: 4.4–8.0 min). The significant effects of the type of beverage and the clarification have been explained earlier in this section. As expected, the mean log D^* values for the concentrates of each fruit were higher than their respective single strength juices (Table 3).

Because of the model design, it was possible to compute for the beverages of each fruit (whether single strength juice or concentrate), the log D^* estimates in case they were clarified or not clarified. In Table 3, three examples are presented for apple, mango and orange. Notice that the meta-analytical model allows us to estimate *Alicyclobacillus* thermal lethality parameters beyond those originally available in the input data set; and this represents the main capability of this model. For instance, no D-values were available for mango single strength juice, but only for non-clarified mango concentrate (Table 1). However, the meta-analysis model can predict D-values for clarified mango single strength juice, non-clarified single strength mango juice and clarified mango concentrate at different inactivation temperature and matrix pH. The confidence about these extrapolated estimates remains to be tested by other thermal inactivation laboratory experiments; these are, experiments for which D-values were not available in the literature, namely, for mango juice, cupuaçu concentrate, berry concentrate, grape concentrate, tangerine concentrate and passion fruit concentrate.

Using Equation (3), the mean temperature shift required for the thermal destruction curve to move one-log cycle (z_T-value) was summarised for single strength juices (11.23 °C; 95% CI: 9.03–13.42 °C) and concentrates (13.35 °C; 95% CI: 9.89–16.80 °C), which are values that can be generalised to all the population of fruits and primary studies. As explained before, because the interaction temperature × type (Table 2) was significant – hence, the slope of the relationship between log D and temperature lower for concentrates – for all fruits, the estimates of z_T values were higher for concentrates than for single strength juices (Table 4). Once again, notice that, as occurred with the log D^* estimates, predictions of z_T could be produced for *Alicyclobacillus* in types of beverages whose lethality kinetics were not investigated in the primary studies. Nonetheless, such extrapolated z_T estimates were subject to greater uncertainty, reason as to why their confidence intervals were slightly broader. For example, for cupuaçu single strength juice (present in the meta-analytical data), the 95% confidence interval of z_T was 6.96–11.56 °C, while for (the non-investigated) concentrate of cupuaçu, it was 7.33–14.00 °C (Table 4).

The z_T values of the fruit beverages estimated by the meta-analysis model were contrasted to those ('observed' z_T values) reported in the primary studies, and in all cases they were within the confidence interval of the model. For instance, the z_T value of *Alicyclobacillus* reported for mango concentrate in the corresponding primary study, (de Carvalho et al., 2008) was 21.27 °C, while the mean estimate of the meta-analysis model was 23.07 °C with a 95% CI of 13.06–33.08 °C (Table 4). For grapefruit juice, Komitopoulou et al. (1999) found z_T values of 11.60, 11.53 and 10.49 °C at a pH of 3.42, 3.0 and 4.0, respectively, whereas the mean z_T value estimated by the meta-analysis model for grapefruit juice was in agreement at 11.17 °C with a 95% CI of 9.38–12.96 °C. From the model, the lowest mean z_T values belonged to berry juice (8.02 °C; 95% CI: 5.34–10.70 °C) and grape juice (7.95 °C; 95% CI: 5.86–10.06 °C), and these were not statistically different one from the other. Both model's estimates were very close to the observed z_T values for berry and grape juice, both of 7.2 °C, found in Walls (1997) and Splittstoesser et al. (1994), respectively.

To further illustrate the model's accuracy, Fig. 2 shows a comparison of log D, as affected by temperature, between the observed values (directly extracted from the primary studies) and the values predicted by the meta-analytical model for different types of beverages at a fixed pH. In all cases, the lines predicted by the model lay close to the observations. This set of examples also demonstrates the flexibility of the model to describe the same or different slopes and intercepts. For clarified apple juice (Fig. 2; top left), the use of a bacteriocin causes a downward shift in the intercept (diminishes the heat resistance) while the random effects realizations from the two primary studies (apple juice with bacteriocin and without bacteriocin) explain the different slopes. For lemon concentrate (Fig. 2; top right) and tangerine juice (Fig. 2; bottom right), the clarification process causes the downward shift in the intercept whereas there is no change in the slope because the variable 'clarification' did not enter the model in significant interaction with temperature. Notice that the model predictions for clarified

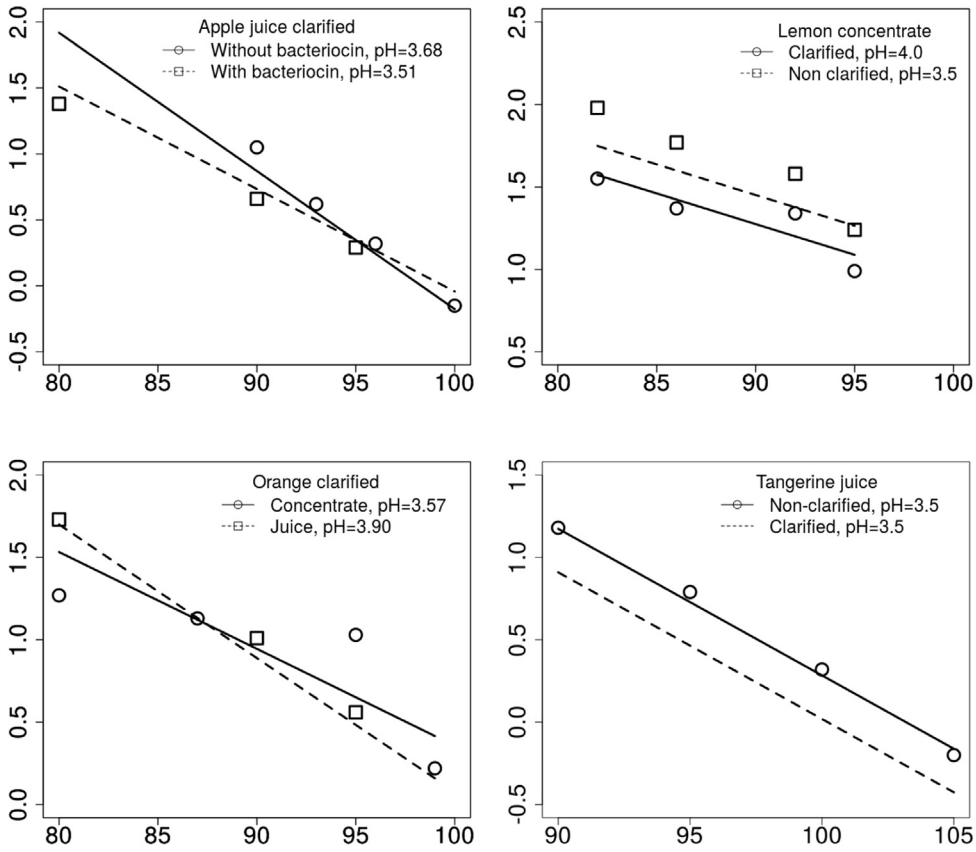

Fig. 2. Relationship between temperature (°C; *x*-axis) and log *D* (*y*-axis), as predicted (lines) by the meta-analysis linear mixed model for different subgroups of types of beverages, in comparison with observed data (markers) when available.

tangerine juice (Fig. 2; bottom right) could not be validated given the absence of thermal resistance data in the literature for this subgroup. For the clarified beverages made of orange (Fig. 2; bottom left), the intercept belonging to the single strength juice is lower than that of the concentrate, and its slope is also affected because of the significant interaction between type of beverage (single strength juice or concentrate) and temperature. Notice that the slope for the single strength juice is steeper than for the concentrate.

In assessing the fitting quality of the meta-analytical model, it was found that the studentised residuals fell between −2.5 and 2.5, and according to the Shapiro–Wilk test, their distribution could be approximated to a normal distribution (not shown). Furthermore, the residuals versus the fitted values (i.e., log *D*) did not exhibit any singular pattern (Fig. 3), as they were randomly spread with a coefficient of correlation of 0.047. In addition, there was good agreement between the fitted and the observed log *D* (Fig. 4) with a high coefficient of correlation of 0.972.

4. Conclusions

Typically, fruit juices will be pasteurized at temperatures around 95 °C for c. 20 s to 2 min (Komitopoulou et al., 1999; Silva and Gibbs, 2001). While the heat treatment alone applied in acidic fruit

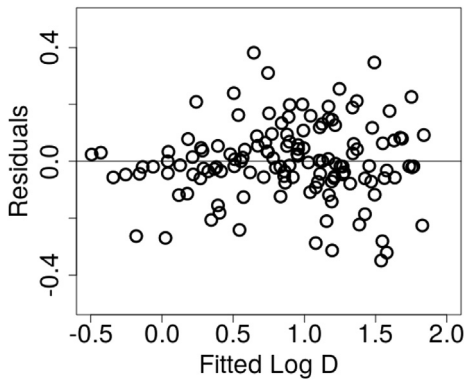

Fig. 3. Relationship between residual values and log *D* fitted by the meta-analytical mixed-effects linear model.

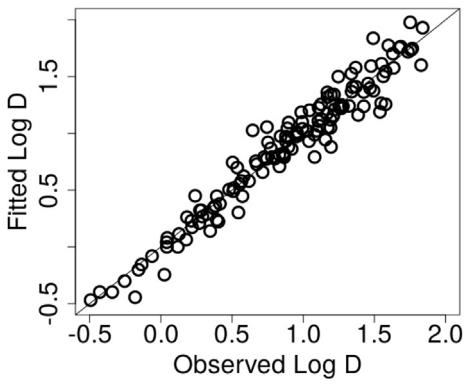

Fig. 4. Relationship between the observed log *D* extracted from the primary studies and the log *D* fitted by the meta-analytical mixed-effects linear model.

products can decrease concentrations of *Alicyclobacillus*, if starting concentrations are high enough, it may not be able to inactivate spores completely. Moreover, as Gouws et al. (2005) pointed out, the heat treatment may even act as a stimulus to germination, which follows outgrowth of the microorganism. The meta-analysis results indicated that the harsh conditions may be insufficient to inactivate the spores of this spoilage microorganism. For instance, the meta-analysis estimated a mean *D*-value of 4.9 min for orange juice at 95 °C and pH 3.5 ($\log D^* = 0.695$; Table 3), suggesting that spores could survive the processing conditions generally used in the fruit beverage industry. Thus, the use of other barriers along with heat treatment to undermine the resistance of *Alicyclobacillus*, such as the addition of bacteriocins prior to pasteurization, may be contemplated. It is known that, even at low levels of 50 IU/ml, the residual nisin would prevent the outgrowth of any surviving spores (Komitopoulou et al., 1999).

Statistical techniques, such as meta-analysis, are very useful to perform a synthesis of a set of distinct but similar experiments. This particular work exemplifies how a common microbiology predictive model such as the Bigelow secondary model can be the basic equation on which a meta-analytical model (i.e., a weighted mixed-effects linear model) is built upon. It is expected that the compilation of the thermal resistance of *Alicyclobacillus* in fruit beverages, carried out in this study, be of utility to food quality managers in the determination or validation of the lethality of their current heat treatment processes. Nevertheless, although the results of this work should in principle provide a summary of the state-of-the-art of *Alicyclobacillus* thermal resistance in fruit beverages, further experimental studies should still be conducted in order to validate the $\log D^*$ and z^* values predicted for some types of beverages, such as mango juice, passion fruit concentrate or grapefruit concentrate, for which there were not available information in the literature.

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